UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

DATE: **December 13, 2019**

SUBJECT: Data Evaluation Record (DER) for a 48-hr Acute Freshwater Invertebrate Toxicity study on *Daphnia magna* (OCSPP 850.1010) using NSPW-L30SS, a Nanosilver Product

PC Code: Generic Silver nanoparticles: 072599 NSPW-L30SS (Nanosilver 005): 072595	DP Barcode No: 447913
Decision No.: 531254	Registration Number: 84610-E
Document Type: DER	Regulatory Action: Registration
MRID: 50617301, 50699401	Case No: 5042

FROM: Kathryn Korthauer, Biologist
Risk Assessment Science Support Branch (RASSB)
Antimicrobials Division (AD) (7510P)

THROUGH: Laura Parsons, Associate Branch Chief
Melissa Panger, Ph.D., Branch Chief
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TO: Aline Heffernan, Risk Manager
Zeno Bain, Product Manager
John Hebert, Branch Chief
Regulatory Management Branch I (RMB I)
Antimicrobials Division (7510P)

RASSB has reviewed the 48-hr Acute Freshwater Invertebrate Toxicity study (OCSPP 850.1010) on *Daphnia magna* using NSPW-L30SS which is referred to as NSPW-Nanosilver in some documents. No analytics were performed within the study. Instead, the registrant requested a crosswalk with the analytics in MRID 50617302 "NSPW-L30SS: Characterization of Dispersion and Dissolution Properties of NSPW_L30SS" (reviewed in DP448178) to determine an approximate measured nanosilver concentration. The Agency has accepted the crosswalk and has calculated an EC_{50} for the nanosilver portion of the NSPW molecule.

The study is considered acceptable, non-guideline and the resulting EC_{50} can be used in the risk assessment.

DATA EVALUATION RECORD (DER)
AQUATIC INVERTEBRATE ACUTE TOXICITY TEST, FRESHWATER DAPHNIDS
(OPPT 850.1010)

Chemical: NSPW-L30SS
PC Code: 072599 Silver Nanoparticles. NSPW-L30SS (Nanosilver 005): 072595

Citation: MRID 50617301. Mikulas, J. (2018) NSPW-L30SS: Daphina magna 48-Hour Acute Toxicity Test. Project Number: 21452/17. Unpublished study prepared by Stillmeadow, Inc. 45p.

MRID 50699401. Mikulas, J. (2018) NSPW-L30SS: Daphnia magna 48-Hour Acute Toxicity Test. Project Number: 21452/17. Unpublished study prepared by Stillmeadow, Inc. 48p.

MRID No: 50617301 and 50699401 (update)
Reviewer: Kathryn Korthauer, Biologist, AD/RASSB
Secondary Reviewer: William Erickson, Biologist. AD/RASSB
Study classification: Acceptable

Results synopsis:
All results presented here are based on calculations involving the nominal concentrations and the analytics presented in MRID 50617302 "NSPW-L30SS: Characterization of Dispersion and Dissolution Properties of NSPW_L30SS". Likewise, nanosilver is abbreviated "Nano Ag" throughout.

Type of Definitive Test	Nominal EC50 (NSPW-L30SS) [1]	Analytical EC50 (Nanosilver portion of the NSPW molecule) [2]
Renewal (at 24 and 48 hrs)	187.5 µg /L NSPW-L30SS	0.150 µg/L Nano Ag
Non-renewal (at 48 hrs)	180.6 µg /L NSPW-L30SS	0.144 µg/L Nano Ag

1- EC50s in study reported as mg/L. 1 mg/L= 1000 µg/L
2- Based on the dissolution study (MRID 50617302) the stock NSPW-L30SS solution (1 g NSPW/1000 mL) contained 26.62% solids of which 0.30% is Nano Ag. Therefore, the EC50 of Nano Ag (analytical) = Nominal EC50 * 0.2662* 0.0030

Study protocol: Protocol for Study 21452-17 with NSPW-L30SS. Protocol was based on OCSPP Guideline 850.1010.

Materials and Methods:

Test substance: NSPW L30SS (Silane) Covalently Bound Elemental Silver. 1.20%. Manufactured Mar 7, 2018. Batch No: 1. Lot #L30SS180307. Expiration date Mar 6, 2019. Liquid at room temperature.

Test substance preparation: On day 0 of the range-finding test, 1.00 g of test substance was dissolved in 1000 mL of dilution water and mixed on a shaker table for approx. 48 hrs. Stock was then diluted and homogenized for 15 min after preparation. On day 1 of the renewal range-

finding test and day 0 and 1 of the definitive test, solution was prepared similarly with 0.5 g test solution was dissolved in 500 mL of dilution water.

Reference substances: Silver Nitrate Manufactured by Alfa Aesar. Lot 10T021. Expiration: Sept 28, 2022.

Silver Nanoparticles. (dispersion- nanoparticles, 10nm particle size (TEM), 0.02 mg/mL in aqueous buffer, contains sodium). Manufactured by Sigma-Aldrich. Lot MKCF1890. Expiration: Apr 24, 2023.

Reference substance preparation: On day 0 of both reference toxicity (non-renewal) tests, 0.5 g of silver nitrate or silver nanoparticles was dissolved in 500 mL of dilution water and mixed for 15 min. Stocks were then diluted and homogenized for 15 min after preparation.

Nominal Test Concentrations: Range-finding: 0.1, 1.0, 10, 100, and 1000 mg/L.
Definitive test: 0.0625, 0.125, 0.25, 0.5, and 1.0 mg/L.

Controls/References: Dilution water only. Silver ion and silver nanoparticle references at 0.1 and 0.5 mg/L (non-renewal only).

Test organism: *Daphnia magna*. Source: STILLMEADOW, Inc culture laboratory. No greater than 24-hrs old at dosing. Daphnids were not fed during the test.

Test Type: Conducted in duplicate, one as renewal and one as non-renewal. This was done for the range-finding and definitive test. Silver reference toxicity tests were non-renewal only.

Renewals: For renewal tests, at approx. 24 -hrs after dosing, daphnids were gently removed from old solution and placed in freshly prepared solutions.

Duration: 48-hour.

Test apparatus: 250 mL glass Erlenmeyer flasks and filled with 200 mL of solution

No. Replicates: For definitive and additional reference test: 3 replicates per test concentration, control, and silver references. 10 daphnids per replicate.

Definitive/Reference Test conditions:
Water hardness (tested at test initiation in 1.0 mg/L solution): 80-90 mg/L $CaCO_3$
Total Organic Carbon (TOC) (tested at test initiation in 1.0 mg/L solution): 33 mg/L C

Tested at dosing, 24-hrs for concentrations with 100% immobility, and 48-hrs for concentrations with surviving organisms.
Temperature: 21-22°C.
pH: 7.2-8.0
DO: 86-98%
Conductivity: 345.7-530.0 µmhos/cm

Photoperiod: 16-hr light/ 8-hr dark

Measurements: 24 and 48 hours after dosing each beaker was examined for immobility and the number of live daphnids was recorded.

Analytical Sampling: No analytical sampling was done within the study due to inability to test nanomaterials in the laboratory. The registrant requested a crosswalk with the analytics in MRID 50617302 "NSPW-L30SS: Characterization of Dispersion and Dissolution Properties of NSPW_L30SS" (see below).

Statistical Analysis: The NOEC (No Observed Effect Concentration) and EC50 (Median Effective Concentration) were determined using ToxCalc Version 5.0™. Linear Interpolation was used for EC50 determination. In addition, statistical analysis on the number of surviving organisms was conducted using a one-way Analysis of Variance (ANOVA) with Tukey-Kramer Multiple Comparison post-test.

Results of Definitive Test:

For the definitive renewal test, the control, 0.0625 mg/L, and 0.125 mg/L NSPW-L30SS had 0.00% immobility. Organisms in the 0.25, 0.5, and 1.0 mg/L NSPW-L30SS concentrations had 100% immobility at 24 hrs.

Table 1: Definitive Renewal Test: Immobility Results of NSPW

Nominal Concentration (mg/L test solution)	# Surviving Daphnids: 0 Hr [A]	# Surviving Daphnids: 24 Hrs	# Surviving Daphnids: 48 Hrs	% Immobility
Control	**30**	**30**	**30**	**0**
0.0625	**30**	**30**	**30**	**0**
0.125	**30**	**30**	**30**	**0**
0.25	**30**	**0**	**0**	**100**
0.5	**30**	**0**	**0**	**100**
1.0	**30**	**0**	**0**	**100**

A- 3 Replicates. 10 daphnids each

For the definitive non-renewal test, the control and 0.0625 mg/L had 0% immobility. Organisms treated with 0.125 mg/L NSPW-L30SS had 10.00% immobility. Organisms treated with 0.25 mg/L NSPW-L30SS had 93.33% immobility at 24-hrs and 100% immobility at 48-hrs. Organisms in the 0.5 and 1.0 mg/L NSPW-L30SS concentrations had 100% immobility at 24 hrs.

Organisms treated with 0.1 and 0.5mg/L silver nitrate had 100% immobility at 24 hrs. Organisms treated with 0.1 and 0.5 mg/L silver nanoparticles had 0% immobility at 48 hrs.

Table 2: Definitive Non-Renewal Test: Immobility Results

Nominal Concentration (mg/L test solution)	# Surviving Daphnids: 0 Hr [A]	# Surviving Daphnids: 24 Hrs	# Surviving Daphnids: 48 Hrs	% Immobility
Control	30	30	30	0
0.0625	30	30	30	0
0.125	30	30	27 [C]	10
0.25	30	2	0	100
0.5	30	0	--	100
1.0	30	0	--	100
Silver Nitrate 0.5 mg/L	30	0	--	100
Silver Nanoparticles 0.5 mg/L	30	30	30	0
Control [B]	30	30	30	0
Silver Nitrate 0.1 mg/L	30	0	--	100
Silver Nanoparticles 0.1 mg/L	30	30	30	0

(--): Not Applicable, all organisms dead
A: 3 Replicates. 10 daphnids each
B: This control was only used for the 0.1 mg/L silver nitrate and silver nanoparticle testing.
C: All immobility occurred within on replicate

The author presented an ANOVA analysis with Tukey-Kramer Multiple Comparison post-test using **NOMINAL values** and determined:
(1) Silver nanoparticles at 0.1 mg/L and 0.5 mg/L had no significant difference in survival compared to control. Likewise, the 0.1 mg/L silver nanoparticle concentration had no significant difference when compared to 0.125 mg/L test substance.
(2) There was significantly greater survival in the 0.5 mg/L silver nanoparticles compared to the test substance at 0.5 mg/L.
(3) Silver nitrate had significantly less survival compared to the control and to the equivalent amount of test substance at 0.1 mg/L and both the test substance at 0.5 mg/L and the silver nitrate at 0.5 mg/L had 0.00% survival.

Analytics within "NSPW-L30SS: Characterization of Dispersion and Dissolution Properties of NSPW_L30SS" (MRID 50617302) Used within the Crosswalk.

The analytics for the daphnid study were performed at the Georgia Tech laboratory due to lack of proper equipment within the Stillwater laboratory. Samples were not shipped after the daphnid test, rather both laboratories used the same water and similar preparation methods to make the NSPW solution. Information on the Georgia Tech analyses are found within MRID 50617302, a detailed review and summary is available in DER (DP448178), and the results relevant to the daphnid study are summarized below:

Upon receipt of the NSPW product from the registrant, the Georgia Tech laboratory dried and analyzed the product and found it contained 0.2662 g/mL (26.62%) solids. The complete make-

up of these solids is unknown but would presumably contain the dried nano material along with dried residues of inerts contained within the product.

One of the analytics the Georgia Tech laboratory ran within the study was a dissolution study on a known 10 mg NSPW/L stock solution (10,000 ppb NSPW). Within this solution, it was found that the average total silver content was 41.25-46.85 ppb during the test duration (0-48 hrs) and over the same time frame, the average ionic silver was 10.68-14.84 ppb. After subtracting the average ionic silver content from the total silver, the remaining average particulate (nano) content was calculated to be 26.41-36.12 ppb. It should be noted that the instruments are good to 2 significant figures, therefore 0.40% of NSPW solids are total silver, 0.10% is silver ion, and 0.30% is nanosilver.

Therefore, within the initial NSPW stock solution of this daphnid study (1 g NSPW/1000 mL water), there was 26.62% solids. Of the solids, 0.30% was nanosilver.

Although the timing of the test preparations differed (i.e. daphnids were exposed to the test solution 48 hrs after mixing the solution), the results of the dissolution study show that the concentration of total silver and nanosilver was constant over time.

Calculated Nanosilver EC50s

Based on the analytics within the crosswalk it was determined that for the renewal definitive test, the nanosilver EC50 was 0.150 µg/L Nano Ag. For the non-renewal definitive test, the EC50 was 0.144 µg/L Nano Ag.

Table 3: Calculated EC50 values of NSPW Particles

Test Type	Nominal EC50 (NSPW) [1]	Total Silver EC50 based on analytical recovery from the Dissolution Study [2]	Total Nanosilver EC50 based on analytical recovery from the Dissolution Study [3]
Renewal Definitive Test	187.5 µg/L NSPW-L30SS	0.200 µg/L Total Ag	0.150 µg/L Nano Ag
Non-Renewal Definitive Test	180.6 µg/L NSPW-L30SS	0.192 µg/L Total Ag	0.144 µg/L Nano Ag

1- EC50s in study was reported as mg/L. 1 mg/L= 1000 µg/L

2- Based on the dissolution study (MRID 50617302) the stock NSPW-L30SS solution (1g NSPW/1000 mL) contained 26.62% solids of which 0.40% was Ag (total). EC50 of Total Ag (analytical) = Nominal EC50 * 0.2662 * 0.0040

3- Based on the dissolution study (MRID 50617302) the stock NSPW-L30SS solution (1g NSPW/1000 mL) contained 26.62% solids of which 0.30% was Nano Ag. Therefore, the EC50 of Nano Ag (analytical) = Nominal EC50 * 0.2662* 0.0030

Conclusion: For the renewal definitive test, the 48-hour EC50 was determined to be 0.1875 mg/L (187.5 µg/L) NSPW-L30SS. Based on the results of the Dissolution study submitted in MRID 50617302, this results in an analytical concentration EC50 of 0.150 µg/L nanosilver.

For the non-renewal definitive test, the 48-hour EC50 was determined to be 0.1806 mg/L (180.6 µg/L) NSPW-L30SS with a 95% confidence limit of 0.14-0.20 mg/L. Based on the results of the

Dissolution study submitted in MRID 50617302, this results in an analytical concentration of 0.144 µg/L nanosilver.

Uncertainties

The study author mentioned precipitation of NSPW when added to water. The dissolution study mentioned the presence of a precipitate as well. While the dissolution study was analyzing the quantity of silver in the supernatant portion of the solution (and therefore taking into account the precipitate), it is unknown if the quantity of precipitate was more than, less than, or equal to the precipitate in the daphnids study. For the purpose of risk assessment, it is assumed that the quantity of nanosilver particles in the supernatant was equal to the quantity bioavailable to the daphnids within this study.

Additionally, the analytical concentration of silver in the positive controls is unknown. Therefore, it is unknown what concentration of silver the daphnids in the positive controls were realistically exposed to. Therefore, the results of the ANOVA should be utilized qualitatively.